FRUIT BUD FORMATION AND DEVELOPMENT IN THE CONCORD GRAPE

by

CHRIS RAY BRADLEY

B. S., Kansas State College, 1927

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1933

TABLE OF CONTENTS

INTRODUCTION

The purpose of this investigation is to determine the time of formation and development of the floral parts of the Concord grape. The exact time of fruit bud formation in the grape has received but little study. Fruit growers should know when the flower clusters are initiated within the bud so they can follow proper cultural practices, which include fertilization, cultivation, pruning and protection from pests, knowledge that must be applied a year in advance of the season in which the grapes are harvested.

The practical importance of this problem is apparent. A weakly vegetative cane has buds of low fruiting capacity. A strongly vegetative cane also has buds of low fruiting capacity. An intermediate cane has buds of maximum fruiting capacity. At the time of fruit bud formation the grower must be familiar with the conditions of his vineyard to insure the formation and development of an adequate number of highly productive fruit buds. The proper formation and development of these buds must precede the production of a profitable crop the following year.

LITERATURE REVIEW

Gladwin (5) states that the grape bud, also called an eye, is a compound one, consisting of three distinct buds enclosed within the same scales; the primary or fruit producing bud; the secondary, a wood or sterile bud but sometimes a fruit bearing one, and the teritary bud which is an undeveloped wood bud that expands as a shoot only when the others in the same eye have been injured or destroyed. The primary bud is the principal fruit bearing organ but it may develop into a shoot only. The secondary produces very little fruit and such fruit bearing usually takes place only when the primary bud from the same eye has failed to grow or has been killed as a shoot by frost.

According to Partridge (7) the relation of the diameter of the cane to its fruitfulness shows that diameter can be used as a criterion in the selection of fruiting wood and that "pencil size" canes are more fruitful than other types of canes.

Schrader (9) says that under a given vegetative condition the following relations between the growth in length of a cane and its fruitfulness will prevail:

1. Strongly vegetative canes have buds of low fruiting capacity.
2. Moderately vegetative canes have buds of maximum fruiting capacity.
3. Weakly vegetative canes have buds of low fruiting capacity.

Angelo (2) states that the productiveness of a cane increases for canes up to a diameter of 0.9 cm. in the Concord grape.

According to Pickett's (8) investigations of the relation between cane diameter and productiveness of the Worden grape, the canes having diameters of twelve sixty-fourths to thirty sixty-fourths of an inch between the fifth and sixth nodes showed greater variations in yield. The fruit production on individual canes measured between the fifth and sixth nodes varied from fifty-four ounces on a cane measuring twenty-seven sixty-fourths of an inch to two hundred and sixty-six ounces on a cane measuring fifteen sixty-fourths of an inch.

From the evidence presented in the foregoing paragraphs it seems evident that canes approximately one-fourth inch in diameter are the most productive.

Due to the small amount of literature published on fruit bud formation in the grape, other publications con-

cerning fruit bud formation in other fruit plants have been reviewed.

Tuft and Morrow (10) have shown that different kinds of deciduous fruits vary greatly in the time of fruit bud differentiation. Some of the differences are as follows:

Fruit	Date	Variety
Apple	June 11	Gravenstein
Almond	Sept. 1	Nonpareil
Apricot	Aug. 10	Royal
Cherry	July 12	Early Richmond
Peach	Aug. 10	Elberta

In regard to fruit bud development in the strawberry Waldo (11) states that fruit bud differentiation extends over a long period of time in some varieties of strawberries and in others it is relatively short. In Maryland fruit bud differentiation occurred about the same time with all varieties used and was during the months of September and October.

Goff (6) found incipient development during late summer or autumn in the grape though the differentiation was not so complete as with other fruits. He also states, "with fruits borne laterally on shoots of the same season's growth, the fruit bud cannot always be predetermined".

Bioletti (3) observed in California that fruit bud differentiation in the grape, Vitis vinifera, occurs during the season before the buds open.

Andrew (1) found fruit bud development complete by September 20 and that it is a gradual process of development. Buds were not collected before this time therefore his evidence as to the actual time of fruit bud differentiation is not complete. He states that development does not take place during dormancy but the floral parts develop very rapidly before the buds open in the spring.

MATERIALS AND METHODS

Row 7 of the Concord variety of grape trained to the Munson system in the vineyard of the Kansas Agricultural Experiment Station was reserved for this investigation. The cover crop-clean cultivation system of soil management is used. It consists of sowing hairy vetch in August and plowing or disking it under in the early part of May. Throughout the summer the soil is disked frequently to conserve soil moisture through the prevention of weed growth. The vines in this row were not pruned until early April in 1932.

Collecting Material. Thirty to forty buds located

between the second and twelfth nodes on canes approximately one-fourth of an inch in diameter were removed and brought to the laboratory each week. A razor blade was found to be the best implement for collecting the buds. Part of the cane was cut off with each so the basal portion of the bud would not be disturbed. The buds, as soon as cut from the cane, were put in four ounce bottles containing a two per cent solution of formaldehyde and labeled. These bottles were tightly stoppered and stored at room temperatures.

Dehydration. Andrew (1) tried the Chamberlain (4) method of embedding with paraffin but was unable to get the buds properly infiltrated. This was due to the mass of pubescence which was present in the grape bud. The hairs effectually prevented complete infiltration by paraffin. This, of course, eliminated use of the rotary microtome in sectioning the buds so he sectioned by various methods with the hand microtome.

In this experiment before dehydrating the buds they were taken from the bottles and pricked several times with a very fine needle. The purpose of this was to enable water to enter through this mass of hairs. To insure more thorough penetration of water the outer scales and excess basal portion of the buds were removed before the buds were placed in vials containing water. These vials were placed

in a flask which was attached to a vacuum pump registering a vacuum pull equal to a force necessary to raise a mercury column 20 inches. In a short time the buds rose to the top but when the pump was released from the flask they sank to the bottom of the vials. The buds were kept under this vacuum for three hours.

After pumping the air from the buds they were immersed in a solution of hydrofluoric acid and ethyl alcohol for one week using one part of ethyl alcohol to two parts of hydrofluoric acid. This treatment dissolved the silica from the pubescence of the buds which was very necessary for complete dehydration and clearing.

The buds were dehydrated by a method similar to that used by Zirkle (13). By this method the buds are run through a series of mixtures of water, ethyl alcohol and n-butyl alcohol.

The following series was found satisfactory:

	Water parts	Ethyl alcohol parts	Butyl alcohol parts
1.	89	11	0
2.	82	18	0
3.	70	30	0
4.	50	40	10
5.	30	50	20
6.	5	40	55
7.	0	25	75
8.	0	0	100
9.	0	0	100

The buds were left one and one-half hours in each solution except five and nine. They were left over night in each of these to insure more complete dehydration.

Infiltration and Embedding. After all water was extracted and replaced by n-butyl alcohol the buds were infiltrated with paraffin. This was done by filling glass vials about two-thirds full of melted paraffin. After the paraffin hardened the buds were placed on top of it and covered with n-butyl alcohol. The purpose of covering the

buds with n-butyl alcohol was to keep them from drying. The vials were placed in an oven at 52° C. and as the paraffin melted the buds sank to the bottom. Butyl alcohol being lighter than melted paraffin, it did not sink with the buds. This allowed them to come into intimate contact with almost pure paraffin. One change was sufficient, it being made four hours afterwards. The buds were left in the melted paraffin eight hours.

Butyl alcohol in this investigation has an advantage over the high concentrations of ethyl alcohol because it does not harden the woody scales of the bud. An additional advantage is that butyl alcohol is lighter than paraffin at the latter's melting point and when the buds sink, as described earlier, it remains floating on the paraffin, and does not surround the bud.

After the buds were left in the paraffin for eight hours they were embedded in paper trays in paraffin at 52° C. and were then ready to section on the rotary microtome.

The celloidon method of embedding as described by Wetmore (12) was also used very successfully. The buds were dehydrated and prepared for celloidon embedding in the same manner as just described. Very few buds were embedded according to this method because of the greater convenience and equal success of the paraffin method.

Staining. Gram's iodine stain was tried with only fair success. Safranin and Bismark brown were used successfully but the colors given the tissues by the stains were too similar to differentiate between them readily. Safranin and Delafield's haemotoxylin were next used and found to be the most desirable stain. The staining schedule was as follows:

1.	Xylol	10 minutes
2.	Xylol 50 per cent and alcohol 50 per cent	5 minutes
3.	Absolute alcohol	5 minutes
4.	Safranin	6 hours
5.	Wash in water	2 minutes
6.	Wash in 50 per cent alcohol	5 - 10 minutes
7.	Wash in 95 per cent alcohol	2 - 3 minutes
8.	Tap water	2 minutes
9.	Delafield's haemotoxylin	10 minutes
10.	Wash in tap water 3 or 4 minutes after water turns purple	
11.	Alcohol 50 per cent	2 - 5 minutes
12.	Alcohol 95 per cent	2 - 5 minutes
13.	Absolute alcohol	2 - 5 minutes
14.	Xylol	20 minutes or longer
15.	Mount in balsam	

Many thousand sections were cut and examined and 1,200 were stained and mounted on glass slides in balsam.

OBSERVATIONS AND RESULTS

Leaf primordia and flower primordia of the grape at their initiation are formed from the same kind of tissue

and early in their development it is difficult to distinguish one from the other even when microscopic sections of the buds are examined. Later development shows the floral parts very distinctly.

The grape bud as described earlier by Goff (6) is a compound one consisting of two and frequently three distinct buds; the larger bud is called the primary or fruit producing bud. When this primary bud fails to grow the secondary bud will sometimes produce fruit. The secondary bud, however, never produces more than a small amount of fruit.

The primary or fruiting bud produces a shoot containing two or three nodes at which flowers and leaves are produced alternately. This brings a leaf opposite each inflorescence. In some instances, however, the first two nodes do not produce inflorescence.

Sections of buds that were collected July 5, 1931, (Plate II), show the first stages of floral development. The blossom primordia are not clearly differentiated or easily identified at this time but when compared with the position of the floral and leaf parts on a grape shoot it is apparent that both are present. Development is rapid at this time. In the course of seven days the shoot had lengthened materially, and the longitudinal sections showed

the leaf and blossom primordia on the sides of the shoot in the form of blunt protuberances (Plate III).

The elongation of the shoot is very evident during the next two weeks. Plate IV shows the shoot has developed very rapidly. During the first two weeks in August the blossom primordia again increased in size considerably, (Plate V). The primary bud at this time shows more development than the secondary or tertiary buds. There does not seem to be very much change in the development of the bud during the rest of the growing season. The only change is the lengthening of the blunt protuberances on the side of the shoot or the blossom and leaf primordia, (Plate VII).

During the dormant season growth ceases and no marked change takes place until the first of March. By the last part of March considerable growth has been made by the flowering bud. Plate XI shows great lengthening of the flower stalk and the floral parts are very evident in this photomicrograph. The bud from which this section was made was collected just as the bud scales were separating at the beginning of visible spring growth.

Plates XII and XIII are sections taken from the same eye on April 10, 1933. Plate XII shows the recent development of the floral parts of the primary bud while Plate XIII, which is the secondary bud, shows less devel-

opment than the primary bud on July 5, 1931 (Plate II).

Longitudinal section of a Concord grape bud collected July 12, 1931 (X85).

1. Shoot
2. Scale
3. Flower and leaf primordia
4. Xylem tissue
5. Parenchyma tissue
6. Bud pubescence

Plate I

Photomicrograph of a longitudinal section of the Concord grape bud showing the leaf and blossom primordia just after they started growth on the side of the shoot. Notice the thick mass of pubescent hairs at the top of the shoot. July 5, 1931 (X85).

Plate II

This photomicrograph shows very clearly the shoot development and the increase in number of leaf and blossom primordia.

The protection given the growing point by the scales is evident. The scales show parenchyma tissue very distinctly. July 12, 1931 (X85).

Plate III

This photomicrograph was selected to show the great increase in shoot development. August 1, 1931 (X85).

Plate IV

Plate V shows the increase in length of shoot and the increase in length of the flower and leaf primordia. The flower stalk is located on the right of the shoot. August 15, 1931 (X85).

Plate V

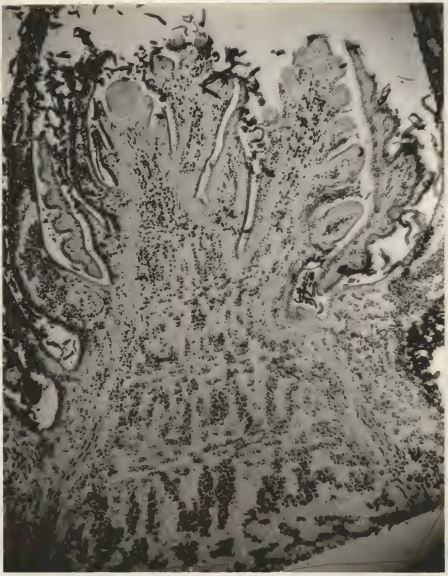
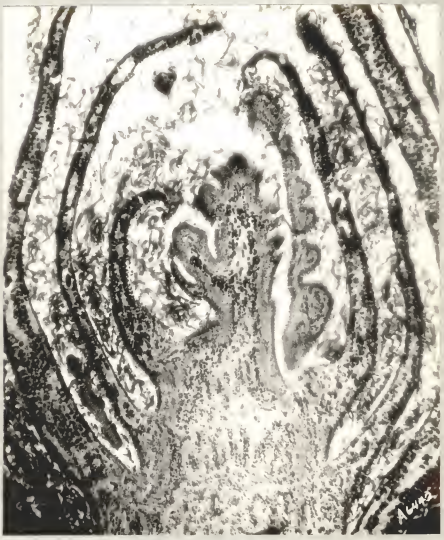


Plate VI shows no further development over Plate V. This is a good example of the decrease in rate of growth as late summer approaches. August 22, 1931 (X85).

Plate VI

This photomicrograph does not show much increase in growth over Plate VI. The flower primordia show a slight increase in development. September 5, 1931 (X85).

Plate VII



This photomicrograph shows the flower and leaf primordia very distinctly. No further development has taken place. October 3, 1931 (X85).

Plate VIII

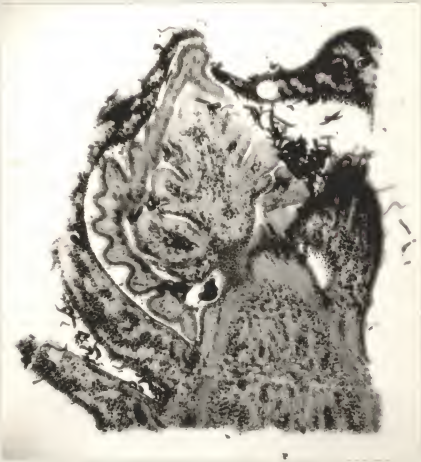


Plate IX shows a photomicrograph with all but one scale removed from the bud. This section was taken from a bud collected during the dormant season while no growth was taking place in any part of the plant. December 19, 1931 (X85).

Plate IX

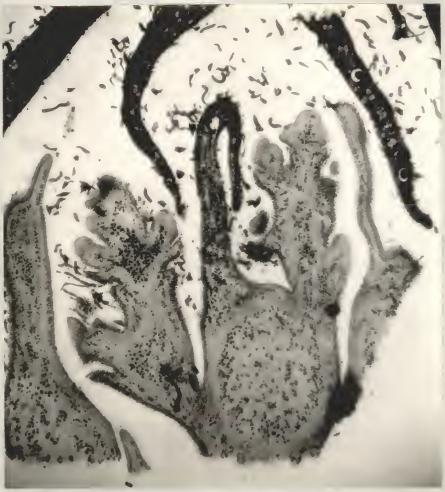


Plate X shows the bud in the same stage of development as the one collected in December. January 23, 1932 (X85).

Plate X

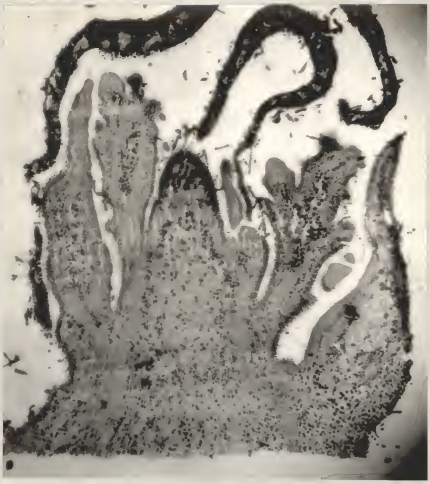
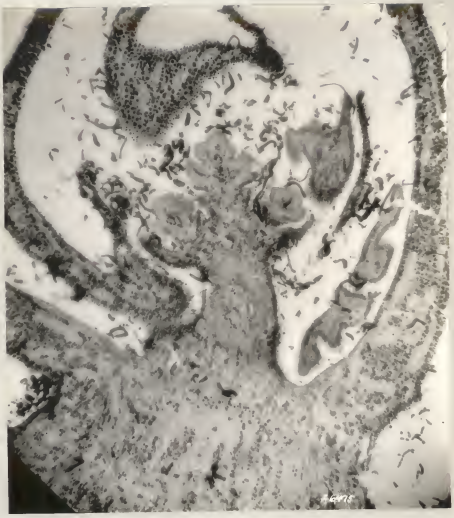


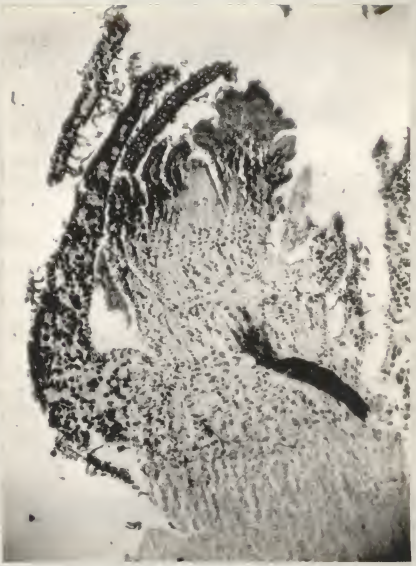
Plate XI shows a photomicrograph of a section of a bud after growth had been resumed in the spring. Note the increased length of flower stalk and the increased floral development. March 31, 1932 (X85).

Plate XI



This photomicrograph is a section of a primary bud collected April 10, 1933. The buds were opening and showed green tips at this time. The left hand protuberance shows the fruit cluster while the right hand one shows the leaf directly opposite (X85).

Plate XII



This photomicrograph shows a section of a secondary bud which was located in the same eye as the primary bud shown in Plate XII. No floral development has taken place in this bud. April 10, 1933 (X85).

Plate XIII

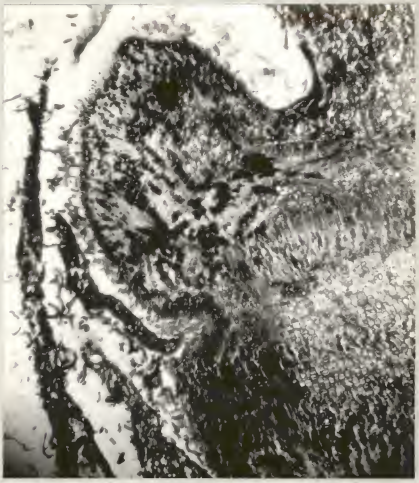


Plate XIV shows a photograph of a Concord grape shoot taken May 1, 1933. Note the arrangement of the leaves and fruiting clusters.

Plate XIV

A study of these photomicrographs shows that the floral development begins approximately between the latter part of June and the first part of July. The axillary bud of the Concord grape is formed about June 15, shortly before the primordia for the following year's crop were initiated. The floral cluster develops quite rapidly during July but during late summer it becomes a more gradually process until the vine becomes dormant. Dormancy took place about the first part of December.

Considering all sections studied it seems certain that fruit bud formation and morphological development ceases as winter approaches. There is no visible change in the development of the floral cluster until the latter part of March when growth is resumed. The photomicrograph of the bud taken March 31, shows the size of the flower clusters just before the bud scales parted.

Very little difference can be observed among the buds located between the second and twelfth nodes on a cane. It is thought that the third to sixth buds should show complete differentiation earlier than those buds beyond these, but as yet there is no definite evidence to support this view.

In many cases the secondary bud was sectioned with the primary bud. The floral clusters were much smaller

than in the primary bud and also appeared to be less developed.

An average of three flower clusters and never more than four was found in one bud.

CONCLUSIONS

1. Fruit bud formation began in the grape between the last of June and the fifth of July in 1931. The bud develops rather rapidly during July but the process is slower in late summer and fall.

2. Fruit bud development does not take place during the winter months while the vines are dormant.

3. The floral parts make rapid development before the buds open in the spring.

4. Pricking the buds with a needle was of great aid in dehydration.

5. Pumping the air from the buds made more complete dehydration possible by replacing the air with water.

6. Hydrofluoric acid dissolved the silica from the bud pubescence which was very necessary for complete dehydration and infiltration by paraffin.

7. A series of mixtures of n-butyl alcohol, ethyl

alcohol and water were found most satisfactory to use for dehydrating and clearing.

8. A mixture of 38° - 40° C. paraffin and 55° C. paraffin at the ratio of one to ten was found satisfactory for infiltrating and embedding.

9. Satisfactory sections were made using the rotary microtome.

10. Safranin and Delafield's haemotoxylin gave the best staining results.

11. The fertility and balance of nutrients in a vineyard soil should be such as to produce medium cane growth at the time of bud differentiation.

ACKNOWLEDGMENT

The author, with the deepest gratitude, wishes to acknowledge the assistance of Professor R. J. Barnett, who suggested the problem and had general oversight of the work; Professor Wm. F. Pickett, who took the photomicrographs and gave valuable information on interpretation of results; also Instructor Margaret Newcomb, Dr. C. L. LePebvre and Professor L. E. Melchers, who aided with the histological work.

LITERATURE CITED

1. Andrew, John A., Jr.
1931. Fruit Bud Formation and Development in the Concord Grape. Unpublished thesis, Kansas State College Library.

2. Angelo, E.
1927. A Study of the Fruiting Behavior of Concord Canes of Various Diameters. Am. Soc. for Hort. Sc. Vol. 24., pp. 155-156.

3. Bioletti, F. T.
1914. Vine Pruning in California. Calif. Agr. Exp. Sta. Bul. 241.

4. Chamberlain, C. J.
1932. Methods in Plant Histology. Fifth Edition. pp. 112-131. University of Chicago Press.

5. Gladwin, F. E.
1917. Cross Morphology of the Grape Bud. N. Y. Agr. Exp. Sta. Bul. 433. p. 121.

6. Goff, E. S.
1901. Investigations of Flower Buds. Wisconsin Agr. Exp. Sta. Rpt. 18. pp. 304-316.

7. Partridge, N. L.
1925. The Fruiting Habits and Pruning of the Concord Grape. Mich. Agr. Exp. Sta. Tech. Bul. 69.

———— 1930. The Fruiting Habits and Pruning of the Campbell Early Grape. Mich. Agr. Exp. Sta. Tech. Bul. 106.

8. Pickett, Wm. F.
1926. A Preliminary Study of the Fruiting Habit of the Worden Grape. Proc. Am. Soc. Hort. Sc. Vol. 23. pp. 135-138.

9. Schrader, A. L.
1926. The Concord Grape - Pruning and Chemical Studies in Relation to the Fruiting Habits of the Vine. Maryland Agr. Exp. Sta. Bul. 286.

10. Tufts, W. P. and Morrow, E. B.
1925. Fruit Bud Differentiation in Deciduous Fruits. Hilgardia Vol. 1. No. 1, May 1925.

11. Waldo, George F.
1930. Fruit Bud Development in Strawberry Varieties and Species. Jour. of Agr. Research. pp. 393-407.

12. Wetmore, R. H.
1932. The Use of Celloidin in Botanical Technic. Stain Technology. Vol. 7. No. 2, April 1932. pp. 37-62.

13. Zirkle, Conway
1930. The Use of N-Butyl Alcohol in Dehydrating Woody Tissue for Paraffin Embedding. Science, Jan. 24, 1930. pp. 103-104.